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US-PAT-NO: 5567601
DOCUMENT-IDENTIFIER: US 5567601 A

TITLE: Subtilisin mutants lacking a primary calcium binding site

DATE-ISSUED: October 22, 1996

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/222; 435/220, 435/221, 435/252.3, 435/252.31, 435/320.1, 435/69.1, 536/23.2

CLAIMS:

What is claimed is:

1. An enzymatically active subtilisin protein which has been mutated to eliminate the ability of said subtilisin protein to bind calcium at a high affinity calcium binding sites wherein the mutated subtilisin protein comprises (1) a deletion of amino acids corresponding to amino acid positions 75-83 of the mature subtilisin BPN', and, (2) one or more additional, stabilizing, mutations comprising amino acid deletions, substitutions, and/or additions in at least one region selected from the following regions and amino acid positions numbered according to the corresponding regions and amino acid sequence positions of the mature subtilisin BPN': the N-terminal amino acid positions 1-8, the .omega.-loop amino acid positions 36-45, the .alpha.-helix amino acid positions 70-74, the helix turn amino acid positions 84-89, and the .beta.-ribbon amino acid positions 202-219.

2. The subtilisin mutant of claim 1, wherein the subtilisin mutant comprises at least one substitution mutation of N218S, M50F, Y217K, P5S, D41A, K43R, K43N, Q271E, Q2K, Q2W, Q2L, A73L, A73Q, Q206C, Q206V, Q206I, Q206W or S3C.

3. The subtilisin mutant of claim 2, wherein the 'subtilisin mutant comprises the substitution mutations of N218S, M50F, Y217K and P5S.

4. The subtilisin mutant of claim 2, wherein the subtilisin mutant comprises the substitution mutations of N218S, M50F, Y217K, Q271E, Q2K, A73L and Q206V.

5. The subtilisin mutant of claim 2, wherein the subtilisin mutant comprises the substitution mutations of N218S, M50F, Y217K, Q271E, Q2K, A73L and Q206C.

6. The subtilisin mutant of claim 2, wherein the subtilisin mutant comprises the substitution mutations of N218S, M50F, Y217K, Q271E, Q2K, A73L, Q206C and S3C.

7. The subtilisin mutant of claim 1, wherein the subtilisin is from a Bacillus strain.

8. The subtilisin mutant of claim 7, wherein the subtilisin mutant is a subtilisin

BPN' mutant, a subtilisin Carlsberg mutant, a subtilisin DY mutant, a subtilisin amylosacchariticus mutant or a subtilisin mesentericopeptidase mutant or a subtilisin Savinase mutant.

9. The subtilisin mutant of claim 8, wherein the subtilisin mutant is a subtilisin BPN' mutant.

10. A recombinant method for producing an enzymatically active subtilisin protein lacking the ability to bind calcium at a high affinity calcium binding site, wherein the mutated subtilisin protein comprises a deletion of amino acids corresponding to amino acid positions 75-83 of mature subtilisin BPN', said method comprising:

(a) transforming a recombinant host cells with an expression vector which comprises a DNA segment or sequence encoding an enzymatically active subtilisin which upon expression provides for the expression of a subtilisin which does not bind calcium and which comprises a deletion of amino acids corresponding to amino acid positions 75-83 of mature subtilisin BPN' and one or more additional, stabilizing, mutations comprising amino acid deletions, substitutions and/or additions in at least one region selected from the following regions and amino acid positions numbered according to the corresponding regions and amino acid sequence positions of the mature subtilisin BPN' : N-terminal amino acid positions 1-8, the .omega.-loop amino acid positions 36-45, the .alpha.-helix amino acid positions 70-74, the helix turn amino acid positions 84-89, and the .beta.-ribbon amino acid positions 202-219;

(b) culturing said host cells under conditions which provide for the expression of the enzymatically active subtilisin mutant; and

(c) recovering the expressed enzymatically active subtilisin mutant from said microbial host.

11. The recombinant method of claim 10, wherein the subtilisin mutant comprises at least one substitution mutation of S221C, N218S, M50F, Y217K, P5S, D41A, K43R, K43N, Q271E, Q2K, Q2W, Q2L, A73L, A73Q, Q206C, Q206V, Q206I, Q206W or S3C.

12. The recombinant method of claim 10, wherein the subtilisin mutant is a subtilisin BPN' mutant.

13. An isolated recombinant DNA which encodes an enzymatically active and stable subtilisin protein, wherein the DNA has been mutated to eliminate the ability of said subtilisin protein to bind calcium at a high affinity calcium binding site, and wherein said mutated DNA which encodes a subtilisin protein comprising (1) a deletion of codons that specify amino acids at positions corresponding to amino acid positions 75-83 of the mature subtilisin BPN' the phrase, and, (2) one or more additional deletions, substitutions and/or additions of codons that specify amino acids at positions in at least one of the following regions and amino acid positions numbered according to the corresponding regions and amino acid sequence positions of the mature subtilisin BPN': the N-terminal amino acid positions 1-8, the .omega.-loop amino acid positions 36-45, the .alpha.-helix amino acid positions 70-74, the helix turn amino acid positions 84-89, and the .beta.-ribbon amino acid positions 202-219.

14. The isolated recombinant DNA of claim 13 which further comprises a codon encoding at least one substitution mutation of S221 C, N218S, M50F, Y217K, P5S, D41A, K43R, K43N, Q271E, Q2K, A73L, A73Q, Q206C, Q206V, Q206I, Q206W or S3C.